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Quantifying diversity losses due to selection for scrapie resistance in three endangered Spanish sheep breeds using microsatellite information

I. Álvarez [a], J.P. Gutiérrez [b], L.J. Royo [a], I. Fernández [a], F. Goyache [a,*]

[a] SERIDA-Somió, C/Camino de los Claveles 604, E-33203 Gijón (Asturias), Spain
[b] Departamento de Producción Animal. Facultad de Veterinaria. Avda. Puerta de Hierro s/n, E-28040-Madrid, Spain

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ABSTRACT

The effect of selection for scrapie resistance on genetic variability in three endangered Spanish sheep breeds (Colmenareña, Mallorquina and Rubia de El Molar) was studied using two different criteria for quantifying contributions to genetic variability: (a) molecular coancestry or genetic identity; and (b) average number of alleles per locus or allelic richness. A total of 236 (81 Colmenareña, 76 Mallorquina and 79 Rubia de El Molar) individuals were genotyped for the PrP gene and for 22 microsatellite markers. The analyses assumed a selective policy aimed at the elimination of the VRQ allele and the reduction of the frequency of the ARQ/ARQ genotype. These goals are approached by rejecting for breeding those individuals with the highest susceptibility for scrapie (risk groups R4 and R5) in a genetic scenario with no previous selection programmes considering the PrP gene polymorphism carried out. When all the individuals classified into risk groups R4 and R5 were removed from the dataset, the total molecular coancestry slightly increased in the Colmenareña breed illustrating that the carriers of undesirable PrP genotypes are not essential to maintain its overall gene diversity. When the allelic richness was considered, the removal of the R4 and R5 individuals gave high losses in the Rubia de El Molar breed. The analyses carried out considering the sex of the individuals informed that most increases in genetic identity in the Colmenareña breed resulted from the removal of the R4 and R5 males while in the Mallorquina breed resulted from the removal of the undesirable females. Losses of diversity in the Rubia de El Molar breed were basically independent of the sex of the individuals due to the balanced contributions to diversity of both sexes. As a general recommendation, not all the individuals of undesirable risk groups should be rejected for reproduction at the same time to avoid irretrievable loses of genetic diversity but according to the sex of the individuals.

1. Introduction

Scrapie is one of the diseases of the group of transmissible spongiform encephalopathies, which include Creutzfeldt–Jakob disease in humans and bovine spongiform encephalopathy (BSE) in cattle (Hunter, 1997; Prusiner, 1998). Different scrapie surveillance and eradication programs have been implemented both in America and Europe (Lynn et al., 2007; Del Rio Vilas and Bohning, 2008). The European Union has decided that breeding programs aimed at decreasing susceptibility to scrapie should be implemented in all the sheep breeds in its territory (European Commission, 2003; see also Gama et al., 2006, for a review). Mutations in the codons 136, 154 and 171 of the third exon of the PrP gene, located on ovine chromosome 13, are related to the degree of susceptibility

* Corresponding author. Tel.: +34 985195303; fax: +34 985195310.
E-mail address: foyache@serida.org (F. Goyache).

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to scrapie in sheep (Hunter, 1997; Elsen et al., 1999; O'Doherty et al., 2002). The European Union has classified the alleles and the genotypes for scrapie in five categories from highly resistant (R1) to highly sensitive (R5). The goals of the breeding programmes must include increasing the frequency of the ARR allele and the ARR/ARR homozygous genotype, which are considered highly resistant to scrapie, and the elimination of the VRQ allele, which has been shown to be highly sensitive to clinical scrapie (Hunter, 1997; Elsen et al., 1999; European Commission, 2003).

Such breeding programmes are criticised because they unavoidably reduce the available genetic variability in a breed, thus affecting selection and conservation programmes in sheep (Álvarez et al., 2007; Windig et al., 2004, 2007). European regulations allow a sequential implementation of these breeding programmes in endangered breeds. However, consumers put traditional breeds kept in non-intensive systems under pressure to offer meat of high quality but also safe to eat. Therefore, rare breeds are driven to apply this kind of breeding programmes that can affect genetic stocks.

Using both simulated and real datasets, different selection regimes have been tested in different sheep breeds to increase scrapie resistance with the minimal costs in terms of genetic variability (Molina et al., 2006; Alfonso et al., 2006; Álvarez et al., 2007). The optimal scenario to test such strategies would include the availability of both genealogical and molecular information (Alfonso et al., 2006; Álvarez et al., 2007; Palhiere et al., 2008). However, pedigrees are usually shallow in rare-endangered breeds. Therefore, molecular polymorphism information is the best option to assess the effect of a selection programme on the genetic variability of a population.

From the 1990s, different approaches for quantifying genetic diversity have been proposed as tools for decision making in genetic conservation programmes, including those focusing on the minimisation of genetic identity assessed via molecular coancestry (Caballero and Toro, 2002; Eding and Meuwissen, 2001) or the average number of alleles per locus (Petit et al., 1998). These methods are based on intuitively appealing criteria to set priorities for conservation: (a) minimisation of the total molecular coancestry is equivalent to the maximization of the total expected heterozygosity or Nei's gene diversity (1987) of a metapopulation (Caballero and Toro, 2002); and (b) the interest in maximising the number of alleles in a set of (sub)populations (Caballero and Toro, 2000, 2002; Fernández et al., 2005) thus including the concept of genetic uniqueness or distinctiveness. However, their use in real scenarios is limited (Glowatzki-Mullis et al., 2008, 2009).

The aim of this research is to asses the effect of selection for scrapie resistance on the within-breed genetic variability in three endangered Spanish sheep breeds, Colmenareña, Mallorquina and Rubia de El Molar, using microsatellite polymorphism. The study assumed a selective policy aimed at the elimination of the VRQ allele and the reduction of the frequency of the ARQ/ARQ genotype in the studied populations. Losses of genetic variability will be assessed via contributions to gene diversity and allelic richness. Consequences of general interest for management of rare sheep populations will be discussed.

2. Materials and methods

2.1. Sampling and genetic analyses

Three endangered Spanish sheep breed, Colmenareña, Mallorquina and Rubia de El Molar, were sampled. The Colmenareña and Rubia de El Molar breeds are located in Madrid (central Spain) while the Mallorquina is the major Balearic sheep breed. Full descriptions of the breeds can be found in Esteban Muñoz (2003). The corresponding flockbooks included, at the moment of sampling, a total of 4463 Colmenareña heads (kept in 10 different flocks), 5514 Mallorquina heads (kept in 44 different flocks) and 1330 Rubia de El Molar heads (kept in 7 different flocks). Pedigrees were very shallow in the three breeds. The average number of equivalent complete generations computed using the program ENDOG (Gutiérrez and Goyache, 2005) were 0.20, 0.30 and 0.33 for, respectively, the Colmenareña, Mallorquina and Rubia de El Molar breeds. Therefore, available genealogies could not be used to assess genetic diversity

Blood samples from a total of 81 Colmenareña individuals (39 males and 42 females), 76 Mallorquina individuals (21 males and 55 females) and 79 Rubia de El Molar individuals (36 males and 43 females) were randomly sampled in, respectively, 10, 12 and 7 flocks included in the corresponding flockbooks. The 12 Mallorquina flocks sampled were considered a representative nucleus of the breed. The only restriction applied was avoiding the sampling of full sibs. Mean age of the sampled individuals (age ranges in brackets) were: 4.0 ± 1.9 years (6.2–1.0) for the Colmenareña breed, 4.7 ± 2.4 years (10.0–2.1) for the Mallorquina breed and 4.6 ± 3.1 years (8.0–0.9) for the Rubia de El Molar breed.

Total DNA was isolated from blood samples using the Invisorb® Spin Micro DNA Kit (Invitek, Berlin, Germany) according to the manufacturer instructions. A microsatellite set including 22 markers was analyzed on all the individuals (see Table 1). This set was basically the same than that previously used to assess between-populations genetic relationships in sheep by Álvarez et al. (2009). Genotyping was performed on an Automatic Sequencer ABI 310 using the GeneMapper software (Applied Biosystems, Tres Cantos, Madrid, Spain).

PrP genotypes, analysed at the Central Veterinary Laboratory of the Spanish Ministry of Agriculture, were also provided by the corresponding breeders associations. Individual genotypes were classified in five risk groups (Table 2) from R1 (very low) to R5 (greatest risk). A brief description of the characteristics of the risk groups is as follows (European Commission, 2003): R1: very low risk both at the individual and at the progeny level; R2: low risk both at the individual and at the progeny level; R3: low risk at the individual level and not low at the progeny level depending on the genotype of the other parent; R4: scrapie occasionally recorded at the individual level and higher risk than in R3 progeny at the progeny level; and R5: greatest risk both at the individual and at the progeny level

Table 1
Chromosome location (Chr), number of alleles per marker (n), expected heterozygosity (H_e) and polymorphic informative content (PIC) values per marker in the analysed dataset.

Marker	Chr	n	H_e	PIC
BMS2626	2	4	0.418	0.364
FCB128	2	8	0.767	0.734
CP34	3	7	0.757	0.719
OarHH64	4	10	0.473	0.458
McM527	5	10	0.783	0.751
McM53	6	10	0.676	0.633
ILSTS05	7	7	0.591	0.550
BM2504	8	6	0.628	0.559
BM757	9	6	0.717	0.674
ILSTS11	9	8	0.692	0.656
CSSM15	11	7	0.396	0.373
TGLA53	12	11	0.843	0.824
LSCV29	14	13	0.808	0.784
BMS2461	16	10	0.760	0.734
BM8125	17	6	0.693	0.660
McMA26	18	15	0.839	0.823
INRA26	19	3	0.190	0.177
BMS1948	21	5	0.564	0.479
CSSM31	23	18	0.879	0.869
BMS2843	24	7	0.685	0.623
RBP3	25	7	0.726	0.682
CSSM43	26	16	0.869	0.856

2.2. Breeding strategies

Breed, sex and PrP genotype were considered to define breeding groups for genetic analyses assessing losses of genetic variability due to selection against susceptibility to scrapie. Within breeds, the fitted groups were (number of individuals between commas): (a) low risk males (Colmenareña, 24, Mallorquina, 10, and Rubia de El Molar, 19); (b) low risk females (Colmenareña, 25, Mallorquina, 27, and Rubia de El Molar, 16); (c) high risk males (Colmenareña, 15, Mallorquina, 11, and Rubia de El Molar, 17); and (d) high risk females (Colmenareña, 17, Mallorquina, 28, and Rubia de El Molar, 27).

The low risk groups included those individuals with PrP genotypes classified between risk groups R1 and R3 while the high risk groups included those individuals with PrP genotypes classified between risk groups R4 and R5. This definition was based on the following criteria: (a) European rules aimed at increasing the frequency of the genotype ARR/ARR and elimination of the VRQ allele (European Commission, 2003); and (b) the interest of the breeders associations in reducing the frequency of the ARQ/ARQ genotype, since the ARQ allele has been shown to be associated with the highest risk of BSE in sheep (Baylis, 2002), thus reducing the risk to human health. These goals can only be approached by rejecting for breeding those individuals included in the risk groups R4 and R5.

2.3. Statistical analyses

The program GENEPOP v. 1.2 (Raymond and Rousset, 1995) was used to compute the deviations from the Hardy-Weinberg proportions at marker and population levels. Linkage disequilibrium was tested for the markers on the same chromosome using also the program GENEPOP.

Most statistical analyses were carried out using the program MolKin (Gutiérrez et al., 2005a; current version v3.0 freely available at http://www.ucm.es/info/prodanim/html/JP_Web.htm). See the User's Guide of the program MolKin for a detailed description of the methodologies used. The following parameters characterising genetic diversity were computed at the breed level: heterozygote deficiency due to population subdivision (F_{IS}; Nei, 1987) and raw and adjusted for sample size (rarefacted; Hurlbert, 1971) average number of alleles per locus. Throughout the text, rarefaction analyses were fitted to 18 copies (2-fold the lowest sampling size of a breeding group) to allow a direct comparison among results.

Contributions of the breeds and breeding groups (or their combinations) to diversity were assessed following Caballero and Toro (2002) and Petit et al. (1998).

Table 2
Classification of PrP genotype in risk groups, sample size per breed and sex of the individual, genotype frequencies and allelic frequencies for the PrP gene in three endangered Spanish sheep breeds. Frequencies are given in absolute values and as percentages (in brackets).

	Risk group	Breed						Totals
		Colmenareña		Mallorquina		Rubia de El Molar		
		Males	Females	Males	Females	Males	Females	
Sample size		39	42	21	55	36	43	236
Allele								
AHQ		0 (0)	1 (1.2)	1 (2.4)	5 (4.5)	0 (0)	0 (0)	7 (1.5)
ARH		1 (1.3)	1 (1.2)	1 (2.4)	6 (5.5)	5 (6.9)	0 (0)	14 (3.0)
ARQ		52 (66.7)	48 (57.1)	23 (54.7)	56 (50.9)	47 (65.3)	66 (76.74)	292 (61.9)
ARR	Highly resistant	25 (32.0)	30 (35.7)	12 (28.6)	33 (30.0)	20 (27.8)	18 (20.93)	138 (29.2)
VRQ	Highly sensitive	0 (0)	4 (4.8)	5 (11.9)	10 (9.1)	0 (0)	2 (2.33)	21 (4.4)
Genotype								
ARR/ARR	R1	1 (2.6)	5 (11.9)	2 (9.5)	7 (12.7)	1 (2.8)	2 (4.6)	18 (7.6)
ARR/AHQ	R2	0 (0)	1 (2.4)	0 (0)	2 (3.6)	0 (0)	0 (0)	3 (1.3)
ARQ/AHQ	R3	0 (0)	0 (0)	1 (4.8)	3 (5.5)	0 (0)	0 (0)	4 (1.7)
ARR/ARH		0 (0)	0 (0)	1 (4.76)	3 (5.5)	2 (5.6)	0 (0)	6 (2.5)
ARR/ARQ		23 (59.0)	19 (45.2)	6 (28.6)	12 (21.8)	16 (44.4)	14 (32.6)	90 (38.1)
ARR/VRQ	R4	0 (0)	0 (0)	1 (4.8)	2 (3.6)	0 (0)	0 (0)	3 (1.3)
ARQ/ARQ		14 (35.8)	14 (33.3)	8 (38.1)	19 (34.6)	14 (38.9)	26 (60.5)	95 (40.2)
ARQ/ARH		1 (2.6)	1 (2.4)	0 (0)	3 (5.4)	3 (8.3)	0 (0)	8 (3.5)
VRQ/VRQ	R5	0 (0)	2 (4.8)	2 (9.5)	4 (7.3)	0 (0)	1 (2.3)	9 (3.8)

Caballero and Toro (2002) proposed to set priorities for conservation using as criterion the maintenance of the maximum overall Nei's (1987) gene diversity (*GD*) in the preserved set of breeds. Note that this is equivalent to minimise the overall molecular coancestry ($\bar{f}$), or genetic identity, because $GD = 1 - \bar{f}$. The average molecular coancestry over a entire metapopulation ($\bar{f}$) consisting of n subpopulations, subpopulation i with N_i breeding individuals, being f_{ij} the between-subpopulations i and j average coancestry, including all $N_i \times N_j$ pairs of individuals, and f_{ii} the average pairwise coancestry within subpopulation i, is computed as $\bar{f} = \sum_{i=1}^{n}(N_i/N_T)[f_{ii} - \left(\sum_{j=1}^{n} D_{ij}N_j/N_T\right)]$, where D_{ij} is the between-populations Nei's (1987) minimum distance. This formula allows to separate the contributions to the total $\bar{f}$ due to the within breeds diversity (f_{ii}) and the between-breeds genetic distance. Therefore, $\bar{f}_T = \bar{f}_W - \bar{f}_B$, where $\bar{f}_T$ is the total contribution to $\bar{f}$, $\bar{f}_W$ is the contribution to the within-breeds identity and $\bar{f}_B$ the contribution to the between-breeds identity.

Petit et al. (1998) proposed to asses the contribution of the ith population to the total allelic richness as $C_T^g(i) = (\hat{k}_T^g - \hat{k}_{T\setminus i}^g)/(\hat{k}_T^g - 1)$, where $\hat{k}_T^g$ is the Hurlbert's (1971) estimator of the total allelic richness in the whole analysed population, $\hat{k}_{T\setminus i}^g$ is the estimator of the total allelic richness when the ith population is excluded. The partitioning of $C_T^g(i)$ in two components $C_S^g(i)$, which is the contribution to the total allelic richness due to the own allelic richness of the ith population, and $C_D^g(i)$, which is the contribution due to its divergence, can be obtained as $C_S^g(i) = (1/n)((\hat{k}_i^g - \hat{k}_{S\setminus i}^g)/(\hat{k}_T^g - 1))$, where $\hat{k}_{S\setminus i}^g$ is the average rarefacted k after removal of population i, and $C_D^g(i)$ simply by difference $C_D^g(i) = C_T^g(i) - C_S^g(i)$.

Losses of genetic variability will be quantified using the following approaches: (a) sequentially removing all the individuals of a given breed from the whole dataset; (b) at the within-breed level, sequentially removing each high risk group (defined using the sex of the individuals) from the dataset; and (c) at the within-breed level, sequentially removing all the high risk individuals of a given breed from the dataset regardless their sex.

3. Results

Parameters characterising the informative ability of the 22 microsatellites used are given in Table 1. The number of alleles per locus varied from 3 (INRA126) to 18 (CSSM31) and the expected heterozygosity and PIC varied, respectively, from 0.190 and 0.177 (INRA26) to 0.883 and 0.872 (CSSM66). No consistent within-breed deviations from Hardy–Weinberg proportions were detected. No linkage disequilibrium was assessed for the markers located in the same chromosome. Since no microsatellites located on ovine chromosome 13 were used, linkage with PrP polymorphism was not considered. Overall, this scenario characterises the genotyped microsatellite set as a useful tool to obtain the goals of this research.

Genotypic and allelic frequencies on the PrP gene for the sampled individuals are given in Table 2. The ARQ allele frequency was roughly 62%. The second most frequent allele was the favourable ARR (with a frequency of roughly 30%), while the undesirable VRQ allele was present at a frequency of 4.4%. Nine out of 15 possible PrP genotypes were identified in the sampled individuals (only 6 in the Colmenareña and Rubia de El Molar breeds). From the identified PrP genotypes, 40.2% were ARQ/ARQ, which is classified at the undesirable risk level R4, and 38.1% were ARR/ARQ (risk level R3). Moreover, the beneficial ARR/ARR genotype is only present in 7.6% of individuals (1 Colmenareña, 2 Malllorquina and 1 Rubia de El Molar males).

Molecular parameters characterising the genetic variability in the sampled breeds are given in Table 3. The three breeds showed consistent heterozygote deficiency (characterized by high and positive F_{IS} values) probably caused by strong within-flock founder effects and poor between-flocks gene flow. In addition, rarefacted (to 18 copies) average number of alleles per locus was similar across breeds, varying from 4.0 for Mallorquina to 4.3 for Colmenareña.

Changes in metapopulation diversity were computed after removal each analysed breed to ascertain if overall contributions to diversity are negligible or not (Table 3). The removal of any of the three analysed breeds gave diversity losses regardless the criterion considered was molecular coancestry or allelic richness. Even though the number of breeds analysed here is very limited, this shows that, in principle, efforts for conservation of all these genetic backgrounds could be justified. The highest increase of genetic identity was assessed after removal of the Rubia de El Molar breed (3.2%) while the Colmenareña breed was that contributing the most to overall allelic richness (6.6%). The removal of the Mallorquina breed gave the lowest increase of both overall molecular coancestry (0.4%) and allelic richness (1.4%).

Table 3
Within-breed deficit of heterozygotes due to populations subdivision (F_{IS}), molecular coancestry (f_{ij}) and raw (A) and rarefacted (to 18 copies, see text) for the analyzed dataset. Contributions to overall diversity of the three analysed breeds are also given. Positive contributions to diversity assessed using these criteria mean that the remaining dataset has a lower genetic diversity than the original one and, consequently, the assessed population would be preferred for conservation. Consistently with the text, $\bar{f}_W$, $\bar{f}_B$ and $\bar{f}_T$ mean, respectively, within-population, between-populations and total contributions to molecular coancestry. Also, C_W, C_B and C_T mean, respectively, within-population, between-populations and total contributions to allelic richness.

Breed	F_{IS}	f_{ii}	A	$A_{(18)}$	$\bar{f}_W$	$\bar{f}_B$	$\bar{f}_T$	C_W	C_B	C_T
Colmenareña	0.103	0.362	6.6	4.4	0.5	1.0	1.5	0.3	6.3	6.6
Mallorquina	0.113	0.384	6.5	4.2	−1.0	1.4	0.4	−1.0	2.3	1.4
Rubia de El Molar	0.138	0.361	6.7	4.2	0.6	2.6	3.2	0.7	5.5	6.2
Total	0.118	0.323	8.8	5.0						

Table 4
Quantification of the overall diversity at the within–breed level after removal different breeding groups defined according to PrP genotype and sex of the individuals. Number of individuals included in the breeding groups, contributions (in percentage) to within-group, between-groups and total diversity of different combinations of the high risk breeding groups (defined in the text) are given. Positive contributions to diversity assessed using these criteria mean that the remaining dataset has a lower genetic diversity than the original one and, consequently, the assessed population would be preferred for conservation. Consistently with the text, $\bar{f}_W$, $\bar{f}_B$ and $\bar{f}_T$ mean, respectively, within-population, between-populations and total contributions to molecular coancestry. Also, C_W, C_B and C_T mean, respectively, within-population, between-populations and total contributions to allelic richness.

Removed groups	N	$\bar{f}_W$	$\bar{f}_B$	$\bar{f}_T$	C_W	C_B	C_T
1. Colmenareña—high risk males	15	0.5	0.5	1.0	1.9	−0.8	1.1
2. Colmenareña—high risk females	17	−1.4	0.7	−0.7	−4.1	3.3	−0.8
1 + 2	32	−0.3	0.7	0.4	−1.7	1.7	0.0
3. Mallorquina—high risk males	11	−0.8	0.5	0.3	−2.8	1.9	−0.9
4. Mallorquina—high risk females	28	2.0	0.5	2.5	1.8	−0.7	1.1
3 + 4	39	1.9	0.8	2.7	−0.8	0.3	−0.5
5. Rubia de El Molar—high risk males	17	0.6	0.8	1.4	1.2	0.2	1.4
6. Rubia de El Molar—high risk females	27	0.4	0.7	1.1	1.9	1.0	2.9
5 + 6	44	2.9	1.0	3.9	6.5	1.8	8.3

The contributions to diversity of each of the undesirable breeding groups were assessed within-breed. Analyses were done with and without considering the sex of the individuals (Table 4). The obtained results varied across assessment methodologies. When all the individuals classified into risk groups R4 and R5 were removed from the dataset, the average molecular coancestry increased substantially in the Mallorquina (2.7%) and Rubia de El Molar (4.5%) breeds. The same parameter did not vary to a large extent in the Colmenareña (0.4%) breed. The carriers of undesirable PrP genotypes would not be essential to maintain the overall heterozygosity in this breed. When allelic richness was considered, the removal of the R4 and R5 individuals did not affect variability to a large extent in the Colmenareña (0.0%) and Mallorquina (−0.5%) breeds but gave a high reduction of the average number of alleles per locus in the Rubia de El Molar breed (8.3%). The analyses carried out including the sex of the individuals in the definition of the breeding groups informed that most increases in genetic identity in the Colmenareña breed resulted from the removal of the R4 and R5 males while in the Mallorquina breed resulted from the removal of the undesirable females. Significant losses of diversity arose in the Rubia de El Molar breed regardless the sex of the removed individuals. Both males and females had balanced contributions to diversity within this breed.

4. Discussion

No breeding programmes considering the PrP gene polymorphism were implemented in the analysed breeds before the moment of sampling. In such unselected scenario, the predominant allele in the three breeds was ARQ, which is thought to represent the ancestral form of the PrP gene (Elsen et al., 1999; Drögemüller et al., 2001) and has been shown to be predominant in other Iberian sheep breeds (Álvarez et al., 2006; Gama et al., 2006; Álvarez et al., 2005a,b). The most frequent PrP genotype in the analysed sheep breeds, except for Colmenareña, is ARQ/ARQ, which is classified with an unacceptable risk to scrapie (R4) and the highly sensitive VRQ allele has a non-negligible overall frequency of 4.4%. The most frequent genotype in the Colmenareña breed was the heterozygous ARR/ARQ classified into the risk group R3. Since selection against sensitivity to scrapie needs to be accounted for (European Commission, 2003), this overall scenario suggests the need to implement breeding policies to increase genetic resistance to scrapie in the studied breeds. However, frequencies of PrP genotypes with intermediate to low susceptibility to scrapie (from R1 to R3) comprise more than a half of the sampled population with a proportion of ARR/ARQ heterozygotes representing roughly 40%. These proportions are higher than others previously reported in endangered Spanish sheep such as the Xalda breed (Álvarez et al., 2007), thus allowing the implementation of breeding strategies leading to gradual rather than drastic selection of ARR genotypes.

Recent studies have proposed different selection regimes to increase resistance to scrapie in Spanish sheep. Alfonso et al. (2006), in the non-risk Black-faced Latxa of Navarra dairy sheep breed, proposed a selection regime consisting in using only ARR/ARR reproductive males (or with a small proportion of ARR heterozygote rams), with no selection for PrP genotype in females. Consistently with Alfonso et al. (2006), Molina et al. (2006), in Merino, reported that a selection strategy consisting in genotyping rams and eliminating ARQ/ARQ and VRQ carriers, would be the best strategy to improve the resistance and would cause minimal cost and loss of genetic variability. However, this strategy cannot be explored in our breeds because this would not guarantee the elimination of the VRQ allele. Álvarez et al. (2007), in the rare Spanish Xalda sheep, tested the effect on the genetic variability of the breed of a breeding policy in which only male individuals with genotype ARR/ARR (risk group R1) and female individuals with genotypes classified into the risk groups R1 to R3 (which do not include either the VRQ allele or the ARQ/ARQ genotype) were expected to be used for reproduction. Álvarez et al. (2007) reported that although loss of genetic variability due to selection for scrapie resistance was not dramatic on the female path, the effect of this selection regime would critically affect the genetic stock because the available number of rams resistant to scrapie was very low thus reducing the long-term evolutionary potential of the breed. Álvarez et al. (2007) conclude that mating policies focused to obtain resistant individuals from ARR-heterozygote parents should be carefully implemented before the beginning of such a

selective programme to avoid problems in current conservation programs in rare sheep breeds. In accordance with this, the breeding regime tested here, including all heterozygous ARR/ARQ individuals, can satisfy the major criteria underlying selective programmes aimed at increasing genetic resistance to scrapie.

Our approach is different to that recently reported by Palhiere et al. (2008) that assess the actual impact of selection for the PrP gene on genetic variability of four French sheep breeds. However, in agreement with these authors, the impact of such selection programme varies with each particular initial scenario; in our case PrP frequencies and different contributions to diversity across sexes. Rejecting the use for reproduction of the individuals included in the undesirable risk groups would result in increases of the average molecular coancestry (a decrease of overall expected heterozygosity) in the Rubia de El Molar breed (4.5%, see Table 4) while it would be roughly neutral in the Colmenareña breed and would be 'beneficial' in the Mallorquina breed. The situation in the Rubia de El Molar breed is consistent across sex groups thus informing that the genotypes of the individuals classified as R4 and R5 have a low genetic representation in the population. This is not the case of the other two breeds in which contribution of males and females are on the opposite direction.

When allelic richness is assessed using the Petit et al.'s (1998) method, the obtained results are not the same than those previously observed for gene diversity. It has been widely reported that methods based on either maximizing gene diversity or allelic richness do not always give the same but complimentary information (Caballero and Toro, 2002; Ollivier and Foulley, 2005): the Petit et al.'s method basically assess the uniqueness of the removed population with respect the remaining one while the Caballero and Toros's method also accounts for balanced allelic frequencies. The removal of the R4 and R5 individuals in the Mallorquina breed significantly increased molecular coancestry but slight losses on average number of alleles per locus. Interestingly, after removal of the R4 and R5 Mallorquina males the allelic richness increased, informing that this breeding group has low heterozygosity and does not carry rare within-breed alleles. With respect the Rubia de El Molar breed, previous genetic analyses informed that this population has suffered an intense genetic bottleneck (Álvarez et al., 2004; Álvarez et al., 2005a,b,c). The removal of any group of individuals can produce a dramatic reduction of 'rare' alleles as reflected in the current analyses.

Rejecting for reproduction those individuals classified as undesirable according to their PrP genotype risk group is no neutral in the three studied breeds in terms of conservation of genetic stocks. Both gene diversity and allelic richness are essential parameters for conservation purposes since expected heterozygosity is directly related to the amount of additive genetic variance for quantitative traits and high levels of allelic richness are needed for the long-term evolutionary potential of populations because the limit of selection response is determined by the initial number of alleles regardless of the allelic frequencies (Falconer and Mackay, 1996; James, 1971; Fernández et al., 2005). In the rare Spanish Xalda sheep, Álvarez et al. (2007) proposed the implementation of gradual rather than drastic selection policies for leading to gradual rather than drastic selection for resistance to scrapie. In endangered sheep breeds, parameters in addition to PrP genotypes should be considered to preserve genetic stocks (Álvarez et al., 2008). Mating policies implemented to obtain resistant individuals from parents with low genetic representation in a given breed together with a continual monitoring of the affected populations should be carefully planned before the beginning of such a selective programme. The implementation of germplasm banks in order to manage the risks of novel ovine PrP polymorphisms and losses of genetic potential can be also recommendable (Roughsedge et al., 2006).

5. Conclusions

Methods for quantification of contributions to genetic diversity have been shown here to be useful to assess the effect of a selection programme on the genetic variability of an endangered population prior to its implementation. The two methods assayed provided different complimentary information that should be jointly considered to plan selection strategies including conservation of genetic variability. Although the three analysed populations starts from a quite similar scenario in terms of the allele frequencies and expected heterozygosity, the impact on genetic variability of a selection programme aimed at decreasing susceptibility to scrapie will vary with each particular initial scenario, namely initial PrP frequencies and different contributions to diversity across sexes. As a general recommendation, not all the individuals of undesirable risk groups should be rejected for reproduction at the same time to avoid irretrievable loses of genetic diversity but according to the sex of the individuals. Selection of sex groups can be done according to the particular scenario of each breed. In cases such as that of the Rubia de El Molar breed, the joint rejection for reproduction of individuals of both sexes classified under the undesirable risk groups R4 and R5 would be particularly unacceptable and one of these sex groups (reasonably females) should be used for reproduction during a period ensuring that the genetic variability they represent is till present in further generations.

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